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(54) MEGSIN TRANSGENIC RAT

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a disease model animal for pathological analysis or screening for therapeutic agent of renal failure or diabetes.
SOLUTION: The disease model animal is constituted that expression of megsin or a gene functionally equal to the megsin is enhanced, and to have a phenotype selected from elevation of creatinine values in blood,

hyperglycemia symptom, hypoplasia, albuminuria and neurodegenerative.

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CLAIMS

[Claim(s)]
[Claim 1]
The animal used in disease modeling which consists of the following description (a) and a nonhuman mammal which has (b).
(a) equivalent gene expression is reinforcing on Meg Singh or Meg Singh, and a functional target -- and
(b) Present at least one phenotype chosen from the following phenotype a-e.

a) The rise of the creatinine value in blood
b) Hyperglycemia symptom
c) Hypoplasia,
d) albuminuria -- and
e) Neurodegenerative
[Claim 2]
The animal used in disease modeling according to claim 1 which equivalent gene expression is reinforcing on Meg Singh in the kidney or Meg Singh, and a functional target, and presents them a rise and albuminuria of the creatinine value in blood.
[Claim 3]
The animal used in disease modeling according to claim 1 which equivalent gene expression is reinforcing on Meg Singh in the pancreas or Meg Singh, and a functional target, and presents them a hyperglycemia symptom.
[Claim 4]
The animal used in disease modeling according to claim 1 which equivalent gene expression is reinforcing on Meg Singh in nervous tissue or Meg Singh, and a functional target, and presents them neurodegenerative.
[Claim 5]
The animal used in disease modeling according to claim 1 which equivalent gene expression is reinforcing on Meg Singh in the kidney, the pancreas, and

nervous tissue or Meg Singh, and a functional target, and presents them all of phenotype a-e.
[Claim 6]
The animal used in disease modeling according to claim 1 whose gene equivalent on Meg Singh or Meg Singh, and a functional target is Homo sapiens Meg Singh.
[Claim 7]
The animal used in disease modeling according to claim 6 which is a transgenic animal introduced in the Homo sapiens Meg Singh gene.
[Claim 8]
The animal used in disease modeling according to claim 1 whose animal is a rat.
[Claim 9]
The manufacture approach of the animal used in disease modeling which presents one which is chosen from the following phenotype a-e of phenotypes including the following processes.
a) The rise of the creatinine value in blood
b) Hyperglycemia symptom
c) Hypoplasia,
d) albuminuria -- and
e) Neurodegenerative
(a) The process which introduces a recombination gene equivalent on HITOMEGUSHIN or Meg Singh, and a functional target into the fertilized egg of a rat
(b) The process which chooses the individual holding the foreignness gene introduced among the individuals generated from the fertilized egg of a process (a)
[Claim 10]
Furthermore, the manufacture approach of the animal used in disease modeling containing the following process (c) and (d) according to claim 9.
(c) The process which obtains F1 rat which is made to cross the individual chosen at the process (b), and the rat which does not hold said foreignness gene, and holds a foreignness gene by the hetero
(d) The process which obtains F2 rat which is made to cross F1 rats obtained at the process (c), and holds a foreignness gene by the gay
[Claim 11]
How to evaluate the curative effect over the renal dysfunction accompanied by both a rise of the creatinine in blood including the following process of a test compound, and both [either or].
(1) the process which consists of the following description (a) and a nonhuman mammal which has (b) and which medicates an animal used in disease modeling with a test compound -- and

(a) equivalent gene expression is reinforcing on Meg Singh in the kidney or Meg Singh, and a functional target -- and
(b) Present both the rise of the creatinine value in blood, and both [either or].
(2) The process which detects the operation which eases the renal dysfunction accompanied by both a rise of the creatinine in blood of the animal used in disease modeling which prescribed the test compound for the patient, and both [either or]
[Claim 12]
The screening approach of a compound of having a curative effect over the renal dysfunction accompanied by both a rise of the creatinine in blood including the following process, and both [either or].
(1) The process which evaluates the curative effect over the renal dysfunction accompanied by both a rise of the creatinine in blood of a test compound, and both [either or] by the approach according to claim 11
(2) The process which chooses the high test compound of the curative effect over the renal dysfunction accompanied by both a rise of the creatinine in blood, and both [either or] as compared with contrast
[Claim 13]
The physic constituent for a therapy and/or prevention of the renal dysfunction accompanied by both a rise of the creatinine in blood which contains the compound which can be obtained by the screening approach according to claim 12 as a principal component, and both [either or].
[Claim 14]
How to evaluate the curative effect including the following process over the hyperglycemia of a test compound.
(1) the process which consists of the following description (a) and a nonhuman mammal which has (b) and which medicates an animal used in disease modeling with a test compound -- and
(a) equivalent gene expression is reinforcing on Meg Singh in the pancreas or Meg Singh, and a functional target -- and
(b) Present a hyperglycemia symptom.
(2) The process which detects the operation which eases the hyperglycemia of the animal used in disease modeling which prescribed the test compound for the patient
[Claim 15]
The screening approach of a compound of having a curative effect including the following process over hyperglycemia.
(1) The process which evaluates the curative effect over the hyperglycemia of a test compound by the approach according to claim 14
(2) The process which chooses the high test compound of the curative effect over hyperglycemia as compared with contrast

[Claim 16]
The therapy of hyperglycemia which contains the compound which can be obtained by the screening approach according to claim 15 as a principal component, and/or the physic constituent for prevention.
[Claim 17]
How to evaluate the curative effect including the following process over the neurodegenerative disease of a test compound.
(1) the process which consists of the following description (a) and a nonhuman mammal which has (b) and which medicates an animal used in disease modeling with a test compound -- and
(a) equivalent gene expression is reinforcing on Meg Singh in nervous tissue or Meg Singh, and a functional target -- and
(b) Present a neurodegenerative symptom.
(2) The process which detects the operation which eases the neurodegenerative symptom of the animal used in disease modeling which prescribed the test compound for the patient
[Claim 18]
The screening approach of a compound of having a curative effect including the following process over a neurodegenerative disease.
(1) The process which evaluates the curative effect over the neurodegenerative disease of a test compound by the approach according to claim 17
(2) The process which chooses the high test compound of the curative effect over a neurodegenerative disease as compared with contrast
[Claim 19]
The physic constituent for a therapy and/or prevention of the neurodegenerative disease which contains the compound which can be obtained by the screening approach according to claim 18 as a principal component.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[Field of the Invention]
[0001]
This invention relates to an animal used in disease modeling.
[Background of the Invention]
[0002]
Renal failure (renal failure) is syndrome which makes a fall and hyperazotemia (azotemia) of a kidney function a cardinal sign. Renal failure is divided roughly into acute renal failure (acute renal failure) and chronic renal failure (chronic renal failure). In Homo sapiens, when a creatinine value (male normal values: 0.8 - 1.2 mg/dL) rises to 3 or more mg/dL, it is judged with renal failure. Acute renal failure says the symptoms which make a cardinal sign the hyperazotemia produced as a result of the fall of the kidney function whose symptoms are shown rapidly, or a halt. For example, the fall of the kidney function by circulatory disturbance, the nephrotoxicity matter, an allergic response, etc. causes acute renal failure. Acute renal failure is often accompanied by the oliguria. Generally, progress of acute renal failure is reversible and can expect recovery from renal failure by removing a cause.
[0003]
The symptoms to which a kidney function falls to chronically and advances to renal failure by diabetic nephropathy, glomerulonephritis, the nephrosclerosis, the collagen disease, obstructive uropathy, etc. on the other hand are called chronic renal failure. Progress of chronic renal failure is irreversible and it is difficult to recover symptoms. Dialysis will be started if the uremia symptom by chronic renal failure appears. The dialysis patient who considers a certain renal failure as a cause in Japan exceeded 200,000 people in 2000, and amounts to 219,000 persons at the end of December, 2001.
[0004]
To development of the remedy of renal failure, or the elucidation of the failure device of a kidney function, the symptoms model animal of renal failure is useful. The model animal of renal failure is useful in the elucidation of the failure device of a kidney function, and development of the remedy of renal failure. Some renal failure model animals are well-known by current. When a well-known renal failure model animal carries out the failure of the kidney function artificially, it is obtained, and also a part of model of natural onset nature is examined. It considers as the model which has renal dysfunction, for example, the following model animals are well-known. In the symptoms model of renal failure, that from which a necrosis takes place to a proximal tubule serves as an anemic

symptoms model not few at coincidence in many cases.
[0005]
(1) Acute-renal-failure model
Ischemic acute-renal-failure model: Circulatory failure is produced by bleeding, water and electrolyte loss, a shock, etc., and the condition of having started renal ischemia is called ischemic acute renal failure. An ischemic model carries out fixed time amount lock out of the renal artery chief editor, intercepts a renal blood flow completely, or carries out continuous intravenous drip infusion of the norepinephrine to a renal artery, and is produced by stopping a fixed time amount renal blood flow completely. The symptom of renal failure and restorative progress change with ischemic intervals. Extraction of a pair side normal kidney is also performed by the case (nonpatent literature 1 reference).
[0006]
Nephrotoxicity acute-renal-failure model: Acute renal failure is caused by prescribing for the patient the drug which has nephrotoxicity. Generally, the following compounds are used for production of the model of acute renal failure.
Heavy metal
Mercuric chloride (nonpatent literature 2 reference)
Nitric-acid (or acetic acid) uranium (nonpatent literature 3 reference)
Drugs
Gentamycin (nonpatent literature 4 reference)
Cisplatin (nonpatent literature 5 reference)
In addition to this
Concomitant use of a streptozotocin and methylguanidine (patent reference 1 reference)
[0007]
(2) Natural onset renal failure model
Progressive renal-dysfunction model: Nephrotic syndrome is caused and the model of the nephritis hated to progressive is reported by the rat and the mouse (nonpatent literature 6 reference).
Polycytic-kidney model: C57BL / 6cpk mouse, the KKcy/cy mouse (nonpatent literature 7 reference), the Han:SPRD rat (nonpatent literature 8 reference), etc. are known as a model animal which causes the polycytic kidney which carries out autosomal dominant inheritance.
Interstitial nephritis: A CBA/calcium mouse has the network of the gene of kdkd, and an autoimmune disease shows the symptoms of a chronic interstitial nephritis owing to, and it results in renal failure (nonpatent literature 9 reference).
[0008]
(3) The renal failure model by reproductive cell genetic manipulation
The renal failure model animal by the genetic manipulation of a reproductive cell is also produced. The following model animals are known as a glomerulosclerosis natural onset model.
SV40 transgenic mouse (nonpatent literature 10 reference)
Fawn-Hooded rat (nonpatent literature 11 reference)
BUF/Mna rat (nonpatent literature 12 reference)
Mpv17 transgenic mouse (nonpatent literature 13 reference)
Cyclooxygenase 2 (COX2) knockout mouse (nonpatent literature 14 reference)
TGF-beta transgenic mouse (nonpatent literature 15 reference)
HIV-1 transgenic mouse (nonpatent literature 16 reference)

[0009]
(4) Acquired chronic progressive-kidney-failure model
Originally human glomerulonephritis is an acquired disease. Especially becoming a problem clinically is a nephritis hated to progressive especially. 20,000 or more persons result in dialysis installation according to progressive renal dysfunction every year. Therefore, offer of the model animal from a nephritis to renal failure for research of the pathophysiology of progressive renal dysfunction and development of a cure is desired.
Shibata nephritis: -- glomerular basement membrane -- ****** rare ****** -- saccharification -- nephritogenoside which is protein is prescribed for the patient as nephritis inducement matter (nonpatent literature 17 reference).
Mesangium [anti-] antibody nephritis (Thy[anti-]1.1. antibody frequent administration): It passes through an anti-Thy1.1. antibody to a rat, and if vein administration is carried out, a mesangial proliferation nephritis will be produced. However, this symptom heals spontaneously in about four weeks, and differs from the nephritis of the Homo sapiens who hates to progressive. Then, a nephritis is made prolonged by prescribing an anti-Thy1.1. antibody for the patient frequently, and the model which causes progressive renal dysfunction is produced (nonpatent literature 18 reference).
[0010]
Otherwise, medication renal failure models, such as adriamycin (nonpatent literature 19 reference), puromycin (nonpatent literature 20 reference), and a daunomycin (nonpatent literature 21 reference), are known as a drug-induced chronic-renal-failure model.
[0011]
Partial nephrectmised model: In chronic renal failure, it is thought as a factor which advances renal dysfunction that reduction of the number of functional nephrons is important. From this viewpoint, the animal which gave nephrectomy as that symptoms model is used in many cases. For example, 3/4, 5/6, and 7 / 8 nephrectomy rat are known (nonpatent literature 22 and 23 reference). the renal dysfunction of a symptoms model animal -- after the operation -- it goes on gradually and albuminuria is produced, and a glomerular filtration ratio (GFR) falls to progressive, and develops into uremia.
[0012]
The above symptoms models are known as a renal failure model animal. The complicated special technique is required of production and the model animal produced by the nephrectomy also tends (nonpatent literature 24 reference) to produce artificial individual difference. While five sixths and an advanced nephrectomy model like 7 / 8 nephrectmised have the advantage which can produce a model also with juvenile [with underdeveloped renal artery branching], the survival rate of a renal cortex may change with how to cut, and they cause dispersion in an experimental result. Moreover, in order to avoid the effect of an operation, it is necessary to prepare the rest term of an about one - two weeks after the operation.
[0013]
On the other hand, compared with a surgical model, the renal failure model animal by medication of the manifestation frequency of glomerulosclerosis is low, and needs the prolonged duration of test. For example, in an adriamycin model, it is after 16-week progress that generating of glomerulosclerosis is accepted, and it can check broadly after 24 week (nonpatent literature 25 reference). Moreover, the rate of the onset was also one animal among six animals (nonpatent literature 19 reference). Furthermore in the renal failure model by drugs, the multiple organ failure by over-medication (over dose) may be produced. Therefore, it is difficult for the renal failure model by

administration of drugs to make high frequency generate an advanced lesion with sufficient repeatability.

[0014]

Moreover, in the case of the renal failure model animal by genetic manipulation, an onset stage is late and an incidence rate is also low. It does not pass over the incidence rate in 19 weeks old of a male SV40 mouse to 20%, but a female mouse is still lower (nonpatent literature 10 reference). The Fawn-Hooded rat of an incidence rate is also low, and symptoms are also light (nonpatent literature 11 reference). The BUF rat of advance of symptoms is loose similarly (nonpatent literature 12 reference).

[0015]

On the other hand, production of an animal used in disease modeling is tried also about the diabetes mellitus which is one of the causes of chronic renal failure. Medication, and an exogenous thing besides the natural onset are known by the diabetes-mellitus model animal like the renal failure model. There are the following reports as a diabetic natural onset model animal.

WBN/Kob rat (nonpatent literature 26 reference),

BB rat (nonpatent literature 27 reference),

LETL rat (nonpatent literature 28 reference),

NOD/Shi Jic mouse (nonpatent literature 29 reference) etc.

[0016]

However, there are the following troubles in a natural onset model animal.

The rate of the onset is low in many cases.

It is difficult to obtain the animal which has uniform symptoms.

By the time it presents an experiment, a long period of time is the need.

For example, the onset of a WBN/Kob rat is before and after nine-month age, the onset of a BB rat is after 9 weeks old, and the rate of the onset is 40 - 80%. Moreover, it does not pass over the rate of the onset of a LETL rat to about 30%, but in a NOD/Shi Jic mouse, although the rate of the onset of a female individual is comparatively as high as 70 - 80%, the male rate of the onset stops to 20 - 30%, and sex difference is accepted. Moreover, an onset stage is also as late as female 90 age in day and male 150 age in day.

[0017]

Moreover, it is known that a diabetes-mellitus model animal can be obtained by administration of alloxan (ALX: nonpatent literature 30 reference) or a streptozotocin (STZ: nonpatent literature 31 reference). However, there is a trouble that a diabetic onset mechanism changes with the amount used and the use stages of drugs.

As mentioned above, the suitable renal failure symptoms model animal is not known. Consequently, the drug evaluation method about a kidney function improvement operation is not established, either. Therefore, the present condition is that the way which develops the drugs for treating chronic renal failure is shut.

[0018]

On the other hand, the increment in a neurodegenerative disease serves as a big social problem with aging of population. Such a disease is defined as the onset in an adult, chronic progressive circumstances, clear clinical phenotype, the abnormalities in a specific cell that participate in the subset of neurone, and a thing characterized by the result fatal finally (nonpatent literature 32). As an example of a neurodegenerative disease, an Alzheimer disease, Parkinson's disease, amyotrophic lateralsclerosis, Huntington's chorea, etc. are mentioned. With such a neurodegenerative disease, perfect

recovery cannot be expected but a therapy must be chiefly limited to management of a disease. To the elucidation of symptoms, and development of a remedy, an animal used in disease modeling is indispensable. The following model animals are established about various neurodegenerative diseases.

[0019]

The Alzheimer disease (nonpatent literature 33) by transgenics, amyotrophic lateralsclerosis (ALS) (nonpatent literature 34), Huntington's chorea (nonpatent literature 35), and a prion disease (nonpatent literature 36) mouse. Tay Sachs disease and the Sandhoff's disease (nonpatent literature 37) mouse by homologous recombination. The child ceroid-lipofuscinosis model mouse by the mutation about a metabolic fate (nonpatent literature 38).

[0020]

However, these model animals or the model animal produced by physical destruction and medication of a nerve cell was not what each can satisfy in the point of repeatability or pathological findings. So, research of a neurodegenerative disease is difficult. Therefore, acquisition of a model animal is very important when you understand the pathophysiology of the disease of a nervous system.

[0021]

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[Description of the Invention]
[Problem(s) to be Solved by the Invention]
[0022]
The technical problem of this invention is offer of an animal used in disease modeling useful as a model of renal failure or diabetes mellitus. Moreover, this invention offers a technical problem the screening approach of a compound useful for the therapy of renal failure or diabetes mellitus.
Furthermore, the technical problem of this invention is offer of an animal used in disease modeling useful as a model of a neurodegenerative disease. Moreover, this invention offers a technical problem the screening approach of a compound useful for the therapy of a neurodegenerative disease.
[Means for Solving the Problem]
[0023]
this invention persons isolated Meg Singh as a gene specifically discovered to a mesangial cell (international public presentation number WO 99/No. 15652 official report [indication of invention]). And the transgenic mouse which introduced Meg Singh showed clearly that it is useful as a model animal of mesangial proliferative glomerulonephritis. That is, in the mesangium organization of the Meg Singh TRANS GENIC mouse which greeted about 35-40 weeks old, the deposition of complement and the immune complex which consists of an immunoglobulin is accepted in the hyperplasia of the Tsuguaki cell proliferation which makes a mesangial cell a subject, and a mesangium substrate, and a list, and hardening (segmental sclerosis) of metamerism is observed. This transgenic mouse is useful as a model animal of mesangial proliferative glomerulonephritis at the point which shows the pathological findings of mesangial proliferation nature. However, the view of urinary protein etc. was not accepted in the Meg Singh TRANS GENIC mouse, but the kidney function was normal to it. moreover, the long period of time of 35-40 weeks old is required to the onset of a mesangial nephritis (international public presentation number WO 01/No. 24628 official report ([an indication of invention] --)) Drawing 4 , 5, 6, 7, 8, 9 and 10, MIYATA, tea (Miyata, T.) work, "journal OBU clinical INVESU tee GEISHON (J.Clin.Invest.)", the (United States), 2002, the 109th volume, p.585 -593 reference.
[0024]
It was already shown clearly by this invention persons that Meg Singh's compulsive manifestation in a glomerulus caused mesangial proliferative glomerulonephritis. Based on this knowledge, this invention persons advanced research further about the onset mechanism of the renal dysfunction by Meg Singh. And for an animal of a certain kind, it found out that the rise of the creatinine value in blood, albuminuria, or hyperglycemia was observed at an early stage by the Meg Singh gene expression enhancement in the kidney. Furthermore, the animal which shows these symptoms found out that it was useful as an animal used in disease modeling of renal failure or diabetes mellitus, and

completed this invention. Since the animal used in disease modeling of this invention presents the symptoms of renal failure and also shows the symptoms of diabetes mellitus in high frequency in juvenile, it is useful also as a diabetes-mellitus model animal.
Furthermore, when Meg Singh is made to discover in nervous tissue, since the animal used in disease modeling of this invention shows the symptoms of the progressive behavior disorder in high frequency in juvenile, it is useful also as a neurodegenerative animal used in disease modeling. And the animal which discovered Meg Singh in nervous tissue checked that it could use for screening of the remedy of a neurodegenerative disease, and completed this invention.
[0025]
That is, this invention relates to an application at the following animal used in disease modeling and its production approach, and a list.
[1] The animal used in disease modeling which consists of the following description (a) and a nonhuman mammal which has (b).
(a) equivalent gene expression is reinforcing on Meg Singh or Meg Singh, and a functional target -- and
(b) Present at least one phenotype chosen from the following phenotype a-e.
a) The rise of the creatinine value in blood
b) Hyperglycemia symptom
c) Hypoplasia,
d) albuminuria -- and
e) Neurodegenerative
[2] An animal used in disease modeling given in [1] which equivalent gene expression is reinforcing on Meg Singh in the kidney or Meg Singh, and a functional target, and presents them a rise and albuminuria of the creatinine value in blood.
[3] An animal used in disease modeling given in [1] which equivalent gene expression is reinforcing on Meg Singh in the pancreas or Meg Singh, and a functional target, and presents them a hyperglycemia symptom.
[4] An animal used in disease modeling given in [1] which equivalent gene expression is reinforcing on Meg Singh in nervous tissue or Meg Singh, and a functional target, and presents them neurodegenerative.
[5] An animal used in disease modeling given in [1] which equivalent gene expression is reinforcing on Meg Singh in the kidney, the pancreas, and nervous tissue or Meg Singh, and a functional target, and presents them all of phenotype a-e.
[6] An animal used in disease modeling given in [1] whose a gene equivalent on Meg Singh or Meg Singh, and a functional target is Homo sapiens Meg Singh.
[7] An animal used in disease modeling given in [6] which is the transgenic animal introduced in the Homo sapiens Meg Singh gene.
[8] An animal used in disease modeling given in [1] whose an animal is a rat.
[9] The manufacture approach of the animal used in disease modeling which presents one which is chosen from the following phenotype a-e of phenotypes including the following processes.
a) The rise of the creatinine value in blood
b) Hyperglycemia symptom
c) Hypoplasia,
d) albuminuria -- and
e) Neurodegenerative

(a) The process which introduces a recombination gene equivalent on HITOMEGUSHIN or Meg Singh, and a functional target into the fertilized egg of a rat
(b) The process which chooses the individual holding the foreignness gene introduced among the individuals generated from the fertilized egg of a process (a)
[10] The manufacture approach of an animal used in disease modeling given in [9] which furthermore contains the following process (c) and (d).
(c) The process which obtains F1 rat which is made to cross the individual chosen at the process (b), and the rat which does not hold said foreignness gene, and holds a foreignness gene by the hetero
(d) The process which obtains F2 rat which is made to cross F1 rats obtained at the process (c), and holds a foreignness gene by the gay
[11] How to evaluate the curative effect over the renal dysfunction accompanied by both a rise of the creatinine in blood including the following process of a test compound, and both [either or].
(1) the process which consists of the following description (a) and a nonhuman mammal which has (b) and which medicates an animal used in disease modeling with a test compound -- and
(a) equivalent gene expression is reinforcing on Meg Singh in the kidney or Meg Singh, and a functional target -- and
(b) Present both the rise of the creatinine value in blood, and both [either or].
(2) The process which detects the operation which eases the renal dysfunction accompanied by both a rise of the creatinine in blood of the animal used in disease modeling which prescribed the test compound for the patient, and both [either or]
[12] The screening approach of a compound of having a curative effect over the renal dysfunction accompanied by both a rise of the creatinine in blood including the following process, and both [either or].
(1) Process which evaluates the curative effect over the renal dysfunction accompanied by both a rise of the creatinine in blood of a test compound, and both [either or] by the approach given in [11]
(2) The process which chooses the high test compound of the curative effect over the renal dysfunction accompanied by both a rise of the creatinine in blood, and both [either or] as compared with contrast
The physic constituent for a therapy and/or prevention of the renal dysfunction accompanied by both a rise of the creatinine in blood which contains in [13] and [12] the compound which can be obtained by the screening approach of a publication as a principal component, and both [either or].
[14] How to evaluate the curative effect including the following process over the hyperglycemia of a test compound.
(1) the process which consists of the following description (a) and a nonhuman mammal which has (b) and which medicates an animal used in disease modeling with a test compound -- and
(a) equivalent gene expression is reinforcing on Meg Singh in the pancreas or Meg Singh, and a functional target -- and
(b) Present a hyperglycemia symptom.
(2) The process which detects the operation which eases the hyperglycemia of the animal used in disease modeling which prescribed the test compound for the patient
[15] The screening approach of a compound of having a curative effect including the following process over hyperglycemia.

(1) Process which evaluates the curative effect over the hyperglycemia of a test compound by the approach of a publication to [14]
(2) The process which chooses the high test compound of the curative effect over hyperglycemia as compared with contrast
The therapy of hyperglycemia which contains in [16] and [15] the compound which can be obtained by the screening approach of a publication as a principal component, and/or the physic constituent for prevention.
[17] How to evaluate the curative effect including the following process over the neurodegenerative disease of a test compound.
(1) the process which consists of the following description (a) and a nonhuman mammal which has (b) and which medicates an animal used in disease modeling with a test compound -- and
(a) equivalent gene expression is reinforcing on Meg Singh in nervous tissue or Meg Singh, and a functional target -- and
(b) Present a neurodegenerative symptom.
(2) The process which detects the operation which eases the neurodegenerative symptom of the animal used in disease modeling which prescribed the test compound for the patient
[18] The screening approach of a compound of having a curative effect including the following process over a neurodegenerative disease.
(1) Process which evaluates the curative effect over the neurodegenerative disease of a test compound by the approach of a publication to [17]
(2) The process which chooses the high test compound of the curative effect over a neurodegenerative disease as compared with contrast
The physic constituent for a therapy and/or prevention of the neurodegenerative disease which contains in [19] and [18] the compound which can be obtained by the screening approach of a publication as a principal component.
[Effect of the Invention]
[0026]
The animal used in disease modeling of this invention is producible using a transgenic animal. Since a transgenic animal can supply a homogeneous high model animal easily and so much, it enables the high experiment of precision. And the failure was accepted in the glomerulus epithelial cell and the renal tubule, urinary protein was presented to the animal used in disease modeling of this invention, and the view of typical renal failure was accepted in it. Moreover, the view of a neurodegenerative disease was also observed. Thus, the meaning provided with the model animal faithful to actual symptoms is very large.
[Best Mode of Carrying Out the Invention]
[0027]
In this invention, the animal which reinforced the manifestation of Meg Singh in the kidney can be obtained by introducing the gene concerned and carrying out a forcible manifestation in a mesangial cell. In addition, it can act on the promotor of the endogenous (endogenous) Meg Singh gene of the animal, and the Meg Singh gene expression can also be reinforced by administration of the compound which promotes the Meg Singh gene expression. The acquisition approach of the drugs which act on the promotor of the Meg Singh gene and this promotor is well-known (international public presentation number WO 00/No. 43528 official report [indication of invention]).
[0028]

The animal which reinforced Meg Singh's manifestation in the kidney can be obtained by the production approach of a well-known transgenic animal (for example, refer to in the volume for Motonari Katsuki, a "developmental engineering experiment manual", (Japanese : Kodansha), and 1989 the edited by Japanese Biochemical Society, "a new biochemistry experiment lecture and an animal experiment method" (Japan: Tokyo Kagaku Dojin), and 1991). Below, it states according to the production protocol of a common transgenic animal.

[0029]

Homo sapiens Meg Singh is protein in which a code is carried out by DNA with the base sequence shown in array number:1. The presumed amino acid sequence is shown in array number:2. In this invention, the animal which introduced DNA which carries out the code of the protein which has an equivalent function as a transgenic animal on not only Homo sapiens Meg Singh but Homo sapiens Meg Singh and a functional target can be used. When a manifestation [in / functionally / on this invention and / in an EQC / the kidney of a certain kind of animal] is reinforced, it says having the operation which brings renal failure to the animal concerned. In this invention, the animal which presents renal failure will not be restricted, if it is the animal which discovers the target symptom by enhancement of the manifestation in Meg Singh's kidney. The desirable animal in this invention is a rat.

[0030]

As such protein, Meg Singh's homologue in other kinds can be shown, for example. Structure of for example, Latt Meg Singh and mouse Meg Singh is clarified by this invention person at Meg Singh's homologue (refer to international public presentation number WO 99/No. 15652 official report). An amino acid sequence is shown in array number:3, array number:4, and mouse Meg Singh's base sequence and a list, and an amino acid sequence is shown in Latt Meg Singh's base sequence, and a list array number:5 and array number:6.

[0031]

Moreover, generally a polymorphism is shown that the gene of eukaryote is known for a Homo sapiens interferon gene in many cases. According to this polymorphism, even if it produces the permutation of the amino acid beyond one piece or it in an amino acid sequence, proteinic activity is usually maintained. Moreover, generally it is known for the alteration of the amino acid of one piece or some that proteinic activity will be maintained in many cases. Therefore, array number:2, array number:4, and an array number: The gene which carries out the code of the protein which consists of an amino acid sequence which changed artificially the amino acid sequence shown in either of 6 can be altogether used for this invention, as long as this protein brings a failure to a kidney function or a neurological function.

[0032]

The number of variation or variation part of amino acid in protein are not restricted as long as the function is held. Generally the number of variation is less than 30% or less than less than 20%, for example, 10%. The more desirable numbers of variation are less than 5% of all amino acid, and less than 3%. As the desirable number of variation, less than 2% of all amino acid and less than 1% of all amino acid can be shown especially. The case where the variation of some amino acid is permuted as two or more amino acid is included in equivalent protein functionally [this invention]. Some mean the amino acid of 5 and also 4 or 3 or 2, and further 1.

[0033]

In order to maintain a proteinic function, as for the amino acid permuted, it is desirable that it is the amino acid which has a property similar to the amino acid before a permutation. For example, the amino acid belonging to each group as shows below is amino acid which has the property which was mutually alike within the group. Even if it permutes these amino acid by other amino acid in a group, a proteinic essential function is not spoiled in many cases. It is well-known as technique for changing an amino acid sequence, the permutation of such amino acid being called conservative substitution, and holding a proteinic function.
Nonpolar amino acid: Ala, Val, Leu, Ile, Pro, Met, Phe, and Trp
Electric-charge [non-] nature amino acid: Gly, Ser, Thr, Cys, Tyr, Asn, and Gln
Acidic amino acid: Asp and Glu
Basic amino acid: Lys, Arg, and His
[0034]
The protein which has an equivalent function functionally is hereafter named generically Homo sapiens, Latt, or Meg Singh originating in a mouse, and it indicates as Meg Singh. In addition, even if it is the case where DNA which carries out the code of Meg Singh who originates in a mouse as Meg Singh is introduced into Latt, DNA originating in Latt who introduced artificially is DNA of foreignness. However, in order to screen a compound useful to the therapy agent in Homo sapiens as an animal used in disease modeling of the renal dysfunction accompanied by a rise or hyperglycemia of the creatinine in blood for this transgenic animal, it is advantageous to use Homo sapiens Meg Singh's DNA. It is because possibility that the effect to Homo sapiens Meg Singh can be reflected more faithfully is expectable in the body of a transgenic animal.
[0035]
Moreover, the codon to amino acid is well-known in itself, and the selection is arbitrary. For example, in consideration of the codon usage of the host who uses, a codon can be determined according to a conventional method (Gran Tamm, R (Grantham, R.) work, "a NUKUREIKKU ASHIZZU research (Nucleic Acids Res.)", (Britain), 1981, the 9th volume, p.r43 reference). Therefore, DNA which changed the base sequence suitably is also contained in DNA of this invention in consideration of the degeneracy of a codon. The base sequence of DNA follows a conventional method. At least the section using the primer which consists of an synthetic oligonucleotide which carries out the code of the desired alteration by the specific displacement introducing method (sitespecific mutagenesis) It is changeable (a mark, dee, EFU (it Mark(s))). D. F. work, "proceeding OBU THE National Academy of Sciences and OBU THE united States OBU United States (Proc.Natl.Acad.Sci.U.S.A.)", the (United States), 1984, the 81st volume, p.5662 reference.
[0036]
Furthermore, array number:1, array number:3, and an array number: The DNA is contained in DNA by this invention as long as the protein can hybridize with DNA which includes the base sequence of a publication in either of 5, and a code is carried out [protein] by the DNA brings about the failure of a kidney function. It is thought that the array which can be hybridized in a specific array under stringent conditions has many in which a specific array has the protein which carries out a code, and similar activity. As conditions for washing, as "1xSSC, 0.1% SDS, 37-degree-C" extent and severer conditions, "0.5xSSC, 0.1% SDS, 42-degree-C" extent can be shown, and stringent conditions can usually show "0.1xSSC, 0.1% SDS, 55-degree-C" extent as still severer conditions. In addition, DNA which carries out the code of Meg Singh in this invention

can also use the fragment, as long as a failure is brought to the kidney function of a transgenic animal.

[0037]

DNA which carries out the code of Meg Singh used for production of a transgenic animal in this invention can be obtained by the well-known approach based on the base sequence indicated on these specifications. For example, isolation of cDNA which carries out the code of Meg Singh is possible by screening as a probe DNA which consists of a base sequence which showed the cDNA library of a mesangial cell to array number:1, array number:3, or array number:5. Moreover, DNA which carries out the code of Meg Singh can be amplified by performing PCR by using this cDNA library as mold using the primer set up based on the base sequence shown in array number:1, array number:3, or array number:5. Cloning of the magnification product is carried out based on a well-known approach.

[0038]

As for DNA which carries out the code of Meg Singh, it is advantageous to rearrange and to consider [which connected with the promotor who can be discovered in the cell of the animal which should introduce this gene] as a gene construct. The recombination gene construct of this invention can be built DNA which carries out the code of said Meg Singh to the vector in which cloning is possible using a suitable host, and by inserting a promotor and carrying out cloning to the upstream. As a promotor who can use for this invention, the fowl beta actin promotor who can guide the manifestation of a foreign gene by broad vertebrates, such as a mouse and Latt, can be shown.

[0039]

Moreover, an enhancer is combinable in order to reinforce the manifestation of a foreign gene. For example, it is known that the enhancer originating in CMV will reinforce the manifestation of the foreign gene in mammalian.

In construction of the recombination gene construct which consists of these genes, it can have an enhancer and a promotor and the vector which has arranged the multi-cloning site for foreign gene insertion on the lower stream of a river further can be used. The vector with such structure can be built by the approach as shown in an example based on pCAGGS etc. (for example, Niwa, EICHI (Niwa, H.) work, "a gene (Gene)", the (United States), 1991, the 108th volume, p.193 -200 reference). The rabbit beta globin terminator is arranged on the lower stream of a river of a multi-cloning site, and this vector contributes to improvement in the manifestation effectiveness of the inserted foreign gene.

[0040]

With a suitable restriction enzyme, the recombination gene construct started from said vector is fully refined, and is used for production of a transgenic animal. A transgenic animal is produced by introducing said construct into the germinal cell containing an unfertilized egg, a fertilized egg, a sperm, and its progenitor cell etc. As a cell which introduces a construct, it is the phase of a single cell or an amphicytula, and the thing before 8 cell terms is usually used for the phase of the embryogenesis in generating of a nonhuman mammal, and a twist concrete target. as the introductory approach of a construct -- a calcium phosphate method, an electric pulse method, the RIPOFE cushion method, a condensation method, a microinjection method, and party Kurgan -- law, the DEAE-dextran method, etc. are well-known. Furthermore, a transgenic animal is also producible by uniting with an above-mentioned germinal cell the transformed cell obtained in this way.

[0041]
The cell which introduces a construct can be a cell originating in all the nonhuman vertebrates that can produce a transgenic animal. Specifically, cells, such as a mouse, Latt, a hamster, a guinea pig, a rabbit, a goat, a sheep, Buta, a cow, a dog, or a cat, can be used. For example, in Latt, the fertilized egg which can introduce a construct is recoverable by making Latt of Metz who prescribed the ovulation inducing drug for the patient cross Latt of a normal male. Generally in the Latt fertilized egg, a construct is introduced by the microinjection to male pronucleus. What is considered that the cell which introduced the construct succeeded in installation after culture of night extent in the outside of the body is transplanted to a surrogate mother's oviduct, and a transgenic chimera animal is born. Metz which was made to cross with the male which cut the spermatic duct, and was made into the pseudopregnancy condition is used for a surrogate mother.
[0042]
The produced transgenic chimera animal is made to cross with an animal normal for birth of F1 animal after checking that the foreign gene (DNA which carries out the code of Meg Singh) is included in the genome by analyzing the gene of the somatic cell. At this time, an individual with desirable more many copy numbers is chosen. A multiple copy is included in a part with the genome same [DNA of the foreignness generally introduced as a construct] by the serial. Usually, it is because it leads to a lot of gene expression and a clearer manifestation mold can be expected, so that there are many these inclusion copy numbers. In a somatic cell genome, it can check to a construct that the foreign gene (DNA which carries out the code of Meg Singh) is incorporated in the direction of the right by PCR using a specific primer. Moreover, the relative comparison of a copy number is possible by dot blotting methods.
[0043]
What equips a somatic cell with a foreign gene (DNA which carries out the code of Meg Singh) in F1 animal born as a result of this mating is the transgenic animal which can tell a foreign gene (DNA which carries out the code of Meg Singh) to a reproductive cell with heterozygote (heterozygote). Therefore, what holds a foreign gene (DNA which carries out the code of Meg Singh) to a somatic cell is chosen from F1 animals, and if F2 animal which makes these parents can be made, the homozygote animal (homozygote animal) which holds a foreign gene (DNA which carries out the code of Meg Singh) by the gay will be obtained as F2 animal.
[0044]
As long as DNA of Meg Singh of foreignness is discovered with the kidney, even if it is which generation of these transgenic animals, it can use for the renal dysfunction model animal accompanied by both a rise of the creatinine in blood of this invention, and both [either or]. For example, if Meg Singh of this foreignness is discovered with the kidney even if it is the transgenic animal which holds Meg Singh's DNA by the hetero, it is useful as a renal dysfunction model animal accompanied by both a rise of the creatinine in blood, and both [either or]. That is, this invention offers the manufacture approach of the renal dysfunction model animal accompanied by both a rise of the creatinine in blood including the process which makes DNA of Meg Singh of foreignness discover in the kidney, and both [either or].
Moreover, as long as DNA of Meg Singh of foreignness is discovered by the pancreas, even if it is which generation of these transgenic animals, it can use for the hyperglycemia model animal of this invention. For example, if Meg Singh of this

foreignness is discovered by the pancreas even if it is the transgenic animal which holds Meg Singh's DNA by the hetero, it is useful as an animal used in disease modeling which presents a hyperglycemia symptom. That is, this invention offers the manufacture approach including the process which makes DNA of Meg Singh of foreignness discover in the pancreas of the model animal which presents a hyperglycemia symptom.

[0045]

In addition, in this invention, if DNA of Meg Singh of foreignness can be made to discover by the kidney or the pancreas at least, it can consider as the renal dysfunction model animal accompanied by both a rise of the creatinine in blood of this invention, and both [either or], or the animal used in disease modeling accompanied by hyperglycemia. therefore, DNA of Meg Singh of foreignness -- not necessarily -- a kidney -- specific or the pancreas -- it is not necessary to make it specifically discovered

[0046]

That a transgenic animal has a failure in a kidney function can do damage on a glomerulus to the animal in the condition of not having abnormalities in a glomerulus, and it can check it by comparing with contrast. An animal with clear not having a failure in a kidney function is used for contrast. For example, Latt of a wild type is desirable as contrast.

Generally, the damage on a kidney function can be known by measuring the marker of a kidney function. For example, common kidney function markers, such as albumin in a serum creatinine value or urine, can be used. The measuring method of these kidney function markers is well-known. In addition, morphological change of a glomerulus organization can also be made into the index of damage. For example, by the PAS stain of kidney tissue, the number of mesangial cells and the area of a mesangium substrate can be observed, and extent of proliferative glomerulonephritis can be scored.

[0047]

In addition to the manifestation of Meg Singh in the kidney and the pancreas reinforcing, the animal used in disease modeling of this invention is characterized by presenting a rise, the albuminuria, and the hyperglycemia symptom of the creatinine value in blood. The animal used in disease modeling which has such a description is useful as a model animal of renal failure or diabetes mellitus. That is, this invention offers the manufacture approach of the model animal of renal failure including the process which reinforces a manifestation of Meg Singh in the kidney and the pancreas, or diabetes mellitus.

[0048]

The rise of the creatinine value in blood means that the value of the creatinine in blood increases more nearly intentionally than the normal creatinine value of the animal. For example, Latt's normal creatinine in blood is 0.2 - 0.3 mg/dL. Therefore, Latt who shows the creatinine concentration in blood exceeding this range is presenting the rise of the creatinine value in blood.

[0049]

In a normal animal, the creatinine in blood is filtered by mesangium and excreted in urine. Therefore, the value of the normal creatinine in blood of an animal is always adjusted in the fixed range. With the fall of the function of a glomerulus, a creatinine comes to be accumulated into blood, consequently the creatinine value in blood rises. The creatinine is measured by various approaches as a kidney function marker.

[0050]

Specifically, the measuring method using enzymes, such as creatinine deaminase or creatinine amide hydrase, like an enzyme is widely used as a measuring method of the

creatinine in current blood. By use of the reaction like an enzyme, a creatinine can be measured specifically. Moreover, the chemical measuring method using the Jaffe reaction is also well-known. The Jaffe method uses the phenomenon in which a creatinine reacts with a picric acid in an alkali solution, and serves as a coloring matter compound of an orange-red color.

[0051]

The animal used in disease modeling of this invention can be characterized also with hyperglycemia. It is proved that a certain animal is hyperglycemia when the blood sugar level has crossed the normal range. Since the blood sugar level is changed sharply [the time of hungry, and after a meal], normal values are usually set up about the time of hungry, and each after a meal. As for the blood sugar level at the time of hungry, in the case of Latt, let 99 - 180 mg/dL be normal values. Therefore, the blood sugar level at the time of hungry is judged that the individual exceeding this range has hyperglycemia.

[0052]

It is also known that hyperglycemia can be guessed by measuring the level of saccharification of the protein in blood. for example, the saccharification in blood -- albumin and glycosylated hemoglobin are known as a marker which reflects the blood sugar level in the blood over a long period of time more. That is, the blood sugar level reflects the average level of the blood sugar level of a long period of time [markers / these] to changing sharply by the meal. therefore, saccharification -- the hyperglycemia of an animal can also be checked by making protein into an index.

[0053]

As for the animal used in disease modeling of this invention, in addition to a rise and hyperglycemia of the creatinine value in blood, albuminuria is observed preferably. Albuminuria means the symptom by which the protein level in urine is excreted exceeding the normal values of the animal concerned. The approach of measuring the protein in urine is well-known. for example, biochemical technique, such as a protein error method and a dye binding method, -- the judgment of the quality of urinary protein -- it has spread widely as a quantitative measuring method. The urine test paper based on these measurement principles of these is marketed. Moreover, the measuring method based on a principle more immunological as a measuring method of high sensitivity and the specific quality of urinary protein is put in practical use. In an immunological measuring method, the albumin excreted in urine is detected by the antibody to serum albumin, for example.

[0054]

Usually, the protein excreted in urine is a minute amount. For example, Latt's normal protein level in urine is made into 0 - 40 mg/dL. Therefore, in Latt, when the protein in urine is constantly excreted exceeding this value, it is judged with it being albuminuria. The class of protein excreted in urine is not limited in this invention. Usually, the quality of urinary protein is occupied by the albumin and globulin which are the quality of a main protein of the protein in blood.

[0055]

Moreover, the animal used in disease modeling of this invention presents hypoplasia preferably. Hypoplasia means that the incompetence of growth is observed in the increment in weight, maturation, or formation of an organ compared with a normal individual.

[0056]

The manifestation in Meg Singh's kidney and the pancreas reinforces the animal used in

disease modeling based on this invention, and it presents the rise of the creatinine value in blood, albuminuria, and hyperglycemia. The animal used in disease modeling which has such a description is useful as a model animal of renal failure or diabetes mellitus. These symptoms are in agreement with the patient of type 1 diabetes. Therefore, the animal used in disease modeling of this invention is useful as a model of type 1 diabetes. Type 1 diabetes is also called insulin-dependent diabetes mellitus (Insulin dependent diabetic desease;IDDM), and although it is the diabetes mellitus which happens when lack of an insulin arises in the destruction nature lesion of beta cells of pancreas, in many cases, it is shown that the failure of the secretion of an insulin by the autoimmune reaction to pancreas Langerhans' islet is involving.

[0057]

In this invention, the elimination function of the kidney falls, the creatinine value in blood rises beyond normal, and renal failure means the condition that albuminuria is seen. The cause of renal failure has three kinds, ******, nephrogenic, and ******. ****** is the renal failure by the blood-flow fall of the kidney. Nephrogenic is the renal failure by glomerulosclerosis or the renal tubule failure, and ****** is the renal failure by the elimination becoming impossible, consequently it becoming impossible to be able to begin to filter urine from a glomerulus by **, although urine is generated. In this invention, there is no involvement in the origin and all renal failure is included.

[0058]

The animal used in disease modeling of this invention presents renal failure according to the failure of a kidney function. The animal which was not concerned with the cause of having brought about the renal failure condition, but became renal failure is useful as a model of a renal failure condition. Specifically, various failures brought to a living body by renal failure are observable. Moreover, the therapy approach for easing the failure by renal failure can also be studied using a model animal.

[0059]

Since the model animal of this invention can reproduce a renal failure condition in high frequency for a short period of time, it contributes to the research on prevention or the therapy of the developmental mechanism of renal failure, and renal failure. Specifically, the large-scale drugs screening for the elucidation of the failure device of a kidney function and remedy development of renal failure is realizable with the model animal of this invention.

[0060]

The curative effect of the test compound to the renal dysfunction accompanied by both the rise of the creatinine value in blood which is the symptoms of renal failure or diabetes mellitus, and both [either or] can be evaluated using the animal used in disease modeling of this invention. The evaluation approach by this invention is related with the approach of evaluating the curative effect over the renal dysfunction accompanied by both a rise of the creatinine in blood including the following process of a test compound, and both [either or].

(1) the process which consists of the following description (a) and a nonhuman mammal which has (b) and which medicates an animal used in disease modeling with a test compound -- and

(a) equivalent gene expression is reinforcing on Meg Singh in the kidney or Meg Singh, and a functional target -- and

(b) Present both the rise of the creatinine value in blood, and both [either or].

(2) The process which detects the operation which eases the renal dysfunction

accompanied by both a rise of the creatinine in blood of the animal used in disease modeling which prescribed the test compound for the patient, and both [either or]

[0061]

Moreover, the compound for a therapy for the renal dysfunction accompanied by a rise and albuminuria of the creatinine value in blood can be screened using the renal failure model animal of this invention. The screening approach of this invention can measure the activity which restores the renal dysfunction accompanied by a rise and albuminuria of the creatinine value in blood of a test compound by said evaluation approach, and as compared with the contrast which does not prescribe a test compound for the patient, when said activity chooses a large compound, it can carry it out.

[0062]

In the evaluation approach of this invention, or the screening approach, extent of recovery of renal dysfunction can evaluate a kidney function marker as an index. For example, the following kidney function markers are known. The approach for measuring these kidney function markers is also well-known.

The creatinine value in blood

The urea nitrogen in blood

Hemoglobin in urine

Albumin in urine

beta2in urine-micro globulin

Alpha one acid glycoprotein in urine

[0063]

In addition, since hyperglycemia is observed, the model animal of this invention contributes to the research on prevention or the therapy of the developmental mechanism of hyperglycemia, and diabetes mellitus. Specifically, the drugs screening for remedy development of a hyperglycemia symptom or diabetes mellitus is realizable with the model animal of this invention.

The curative effect of the test compound to the hyperglycemia symptom which is diabetic symptoms can be evaluated using the animal used in disease modeling of this invention. The evaluation approach by this invention is related with the approach of evaluating the curative effect including the following process over the hyperglycemia symptom of a test compound.

(1) the process which consists of the following description (a) and a nonhuman mammal which has (b) and which medicates an animal used in disease modeling with a test compound -- and

(a) equivalent gene expression is reinforcing on Meg Singh in the pancreas or Meg Singh, and a functional target -- and

(b) Present a hyperglycemia symptom.

(2) The process which detects the operation which eases the hyperglycemia of the animal used in disease modeling which prescribed the test compound for the patient

Moreover, the compound for a therapy for a hyperglycemia symptom can be screened using the animal used in disease modeling which presents the hyperglycemia symptom of this invention. The screening approach of this invention can measure the activity which improves the hyperglycemia symptom of a test compound by said evaluation approach, and as compared with the contrast which does not prescribe a test compound for the patient, when said activity chooses a large compound, it can carry it out.

Moreover, in the evaluation approach of this invention, or the screening approach, extent of a diabetic curative effect can evaluate a diabetes-mellitus marker as an index.

More specifically, the curative effect over diabetes mellitus can be known by making the blood sugar level, level of urine sugar, etc. into an index. as the index for furthermore getting to know the curative effect over a diabetes-mellitus symptom -- hemoglobin Hc1 and saccharification -- albumin, fructosamine, a keton body, etc. are mentioned. The curative effect over diabetes mellitus can be evaluated by observing a change of these diagnostic indexes with time.

[0064]

Therefore, before and after prescribing a test compound for the patient, the effectiveness as a remedy of a test compound can be evaluated by comparing the observation result of these indexes. Or if an affiliated transgenic animal is used, the effectiveness between test compounds can also be compared by comparing the observation result of these indexes between animals.

[0065]

Moreover, equivalent gene expression is reinforcing this invention on Meg Singh in nervous tissue or Meg Singh, and a functional target, and it relates to the animal used in disease modeling which presents neurodegenerative. Or this invention provides Meg Singh in nervous tissue or Meg Singh, and a functional target with the manufacture approach including the process which reinforces equivalent gene expression of the animal used in disease modeling which presents neurodegenerative. When the manifestation in the nervous tissue of a certain kind of animal is reinforced, it is told to a gene equivalent on Meg Singh and the functional target in this invention that it has the operation which brings neurodegenerative to the animal concerned. As protein equivalent on Meg Singh and a functional target, the protein which has structure as illustrated previously can be shown. In this invention, the animal which presents neurodegenerative will not be restricted, if it is the animal which discovers the target symptom by enhancement of the manifestation in Meg Singh's nervous tissue. The desirable animal in this invention is Latt. The animal used in disease modeling of this invention presents a neurodegenerative symptom. That is, the animal used in disease modeling of this invention shows one or the phenotype description beyond it by which neurodegenerative diseases, such as an Alzheimer disease, Parkinson's disease, and Huntington's chorea, are characterized, and movement disability, such as advance-degeneracy of systemic coordination and advance-degeneracy of locomotion, and recognition disability, anorexia, etc. are contained in this description.

[0066]

By use of the animal used in disease modeling of this invention, screening assay of the compound for a therapy of a neurodegenerative disease can be carried out. That is, this invention relates to the approach of evaluating the curative effect including the following process over the neurodegenerative disease of a test compound.

(1) the process which consists of the following description (a) and a nonhuman mammal which has (b) and which medicates an animal used in disease modeling with a test compound -- and

(a) equivalent gene expression is reinforcing on Meg Singh in nervous tissue or Meg Singh, and a functional target -- and

(b) Present a neurodegenerative symptom.

(2) The process which detects the operation which eases neurodegenerative [of the animal used in disease modeling which prescribed the test compound for the patient]

Based on the evaluation approach of this invention, the screening approach of the compound for a therapy of a neurodegenerative disease can be enforced. That is, this

invention offers the screening approach of the compound for a therapy of a neurodegenerative disease including the following process.
(1) The process which evaluates the curative effect over the neurodegenerative disease of a test compound by said evaluation approach
(2) The process which chooses the high test compound of the curative effect over a neurodegenerative disease as compared with contrast
[0067]
When a test compound adjusts at least one parameter or symptoms by which a neurodegenerative disease is characterized, such as anorexia and movement coordination deletion, or has the effectiveness over it, it is judged that it has the activity about a neurodegenerative disease. In this invention, the activity about a neurodegenerative disease means decreasing or reinforcing extent of the symptom of a neurodegenerative disease. Therefore, if the screening procedure of this invention is used, remission, mitigation, or the compound removed further can be identified for the compound which adjusts advance of a neurodegenerative disease, and/or the phenotype symptom of a disease by combining with the protein or the peptide which participates in advance of a neurodegenerative disease for example, and adjusting, reinforcing or controlling the activity. Furthermore, the screening procedure of this invention can be enforced also as screening for determining the drug with which the effectiveness over a neurodegenerative condition is lacked.
[0068]
As a test compound used for screening of this invention, nature or a synthetic compound, various organic compounds, nature or the compounded saccharide, protein, a peptide, the manifestation product of a gene library, a cell extract, or a fungus body component can be mentioned, for example. In addition, the antisense nucleic acid which controls Meg Singh's manifestation, and the anti-MEGUSHIN antibody it is expected that Meg Singh's activity control is can also be made into a test compound. The animal used in disease modeling of this invention is medicated with these test compounds in taking orally or parenterally.
[0069]
An animal used in disease modeling can be medicated with a test compound before the onset of the renal dysfunction accompanied by the rise of the creatinine value in blood, or albuminuria, and/or after the onset. Before a phenotype is similarly observed in a hyperglycemia symptom, behind can be medicated at an animal used in disease modeling. However, in order to find out the compound which acts more specifically to each disease, after presenting these symptoms, it is desirable to prescribe a test compound for the patient. A more effective administration stage can also be clarified by changing the timing of administration of a test compound and comparing the operation over renal dysfunction or hyperglycemia.
[0070]
Also when evaluating the curative effect of a neurodegenerative disease, behind can be medicated with a test compound before the animal used in disease modeling of this invention shows the symptoms of a neurodegenerative symptom. The same is said of the ability to change the timing of administration and examine an effective administration stage.
[0071]
The test compound chosen by the screening approach of this invention can be made into the active principle of the physic constituent for a therapy for the rise of the

creatinine value in blood, or restoration of the renal dysfunction accompanied by albuminuria after examining safety, stability, etc. further. Moreover, the compound which can be obtained by the screening approach of this invention can be made into the active principle of the therapy of a hyperglycemia symptom, and/or the physic constituent for prevention. Or the compound which can be obtained by the screening approach of this invention can be made into the active principle of the therapy of a neurodegenerative disease, and/or the physic constituent for prevention.

The physic constituent of this invention can be pharmaceutical-preparation-ized according to a well-known galenical pharmacy-manufacturing method, and can be prescribed for the patient. Moreover, the compound itself which is an active principle can also be directly prescribed for the patient. When pharmaceutical-preparation-izing, a medicine can be prescribed for the patient, combining suitably the medium or support generally used as drugs.

[0072]

Moreover, if the code of this compound is carried out by DNA and it gets, this DNA will be included in the vector for gene therapies, and performing gene therapy will also be considered. Administration can be performed by approaches, such as intraarterial injection, an intravenous injection, the administration in a nasal cavity, administration in a bronchial tube, intramuscular administration, hypodermic administration, internal use, and direct administration to the affected part. Although a dose is changed according to conditions, such as whenever [weight / of a patient /, age, and health], or a medication method, if it is this contractor, it can choose a suitable dose suitably.

[0073]

That is, for example in the animal used in disease modeling of this invention, effective concentration is determined by comparing the curative effect over renal dysfunction among various doses. And a dose to which the concentration of the administration compound in a kidney reaches the effective concentration by each above administration roots is determined experientially. In a general administration gestalt, the dose per weight of 1kg is determined as that from which an active principle is distributed over the whole body. If it is the compound considered that kidney translatability is high based on the analysis result of the pharmacokinetics and metabolism in a laboratory animal, a dose can be set up lower.

[0074]

The physic constituent of this invention is blended with a medium or support in consideration of the dose and administration gestalt which were determined. This contractor is usually performing blending an active principle so that a required dose can be attained. More generally 20g of doses of the physic constituent by this invention can be set to 10micro g-500mg from usual 1microg per weight of 1kg. Moreover, in the case of injections, 1/10 to about [of internal use] 1/100 can be made into the standard of a dose. A dose can be adjusted by performing a still more nearly special dosage form design. For example, in such pharmaceutical preparation, although it can also consider as gradual release-ized pharmaceutical preparation by holding to suitable support, since the physic constituent of this invention can maintain high blood drug concentration, it can set up loadings low.

Although this invention is explained still more concretely as an example below, this invention is not limited to this example.

[Example]

[0075]

[Example 1] recombination gene construct
He is Homo sapiens Meg Singh under control of the CAG promotor who are the enhancer of CMV, and a fowl beta actin promotor's hybrid. The expression vector which discovers cDNA was built as follows. First, in order to permute the base sequence in front of Homo sapiens Meg Singh's initiation codon by the Kozak array (GCCGCC), A sense primer () [B44F:]
5'-ATGGATCCGCCGCCATGGCCTCCCTTGCTGCAGCAAATGCAGAG-3' / array number : 7 and an antisense primer
(H30-R:5'-TATCCTGAGGCAGTGTTAACATGAAG-3' / array number: 8) It uses and he is Homo sapiens Meg Singh. PCR is performed to mold and just before an initiation codon the plasmid (pUC-MEGSIN) (patent reference 2 reference) containing cDNA Homo sapiens Meg Singh permuted by the Kozak array 5' fragment of cDNA was amplified. It is a restriction enzyme about pUC-MEGSIN. It cuts by BamHI and HpaI and he is Meg Singh cDNA. Homo sapiens Meg Singh who removed the fragment of about 180 bp(s) containing an initiation codon, inserted the fragment obtained by PCR, and was permuted by the Kozak array The plasmid with cDNA was built.
[0076]
It is a restriction enzyme about the plasmid after carrying out a transformation to Escherichia coli JM 109 and carrying out cloning to it. The fragment of 1.2kbs which cut by BamHI and HindIII and contain the obtained Meg Singh overall length was refined, and it flush-end-ized using TaKaRa Blunting Kit (TAKARA SHUZO make). pBsCAG-2 () [Kawarabayashi] T, Shoji M, and Sato M, Sasaki A, Ho L, and Eckman CB, Prada C-M, and Younkin SG, Kobayashi T, and Tada N, Matsubara E, and Iizuka T, Harigaya Y, and Kasai K and Hirai S (1996) Accumulation of beta-amyloid fibrils in pancreas of transgenic mice.Neurobiol.Aging 17 and 215-222 After EcoRI's having cut and making it a straight chain, the end was graduated similarly and alkaline phosphatase (TAKARA SHUZO) performed dephosphorization processing. To this plasmid, ligation of the fragment of the above-mentioned 1.2kbs was carried out, it was rearranged, the plasmid was produced, the transformation was carried out to Escherichia coli JM 109, and cloning was carried out to it. Homo sapiens's MEGSIN cDNA inserted directivity selected the same clone as chicken beta-actin promoter by sequencing, and made this recombination plasmid pBsCAG/Megsin (drawing 1 B). pBsCAG-2 [in addition,] pCAGGS () [Niwa H,] [Yamamura] K and Miyazaki J (1991) Efficient selection for high-expression transfectants with a novel eukaryotic vector.Gene 108,193-200, a CMV enhancer, a fowl beta actin promotor, and a rabbit beta globin terminator SalI-PstI fragment which it has It included in the SalI-PstI part of pBluescript II SK (-) and (Invitrogene), and was produced (drawing 1 A).
It is a restriction enzyme about pBsCAG/Megsin. It cuts by ScaI, SalI, and NotI, and he is after [agarose gel electrophoresis] and Meg Singh cDNA. The fragments of about 3.4 included kbs were cut down and collected, and it was used for production of a transgenic rat (drawing 1 B).
[0077]
Production of a [example 2] transgenic rat
Abdominal administration of the PMSG (pregnant-mare-serum Gonatrophin) was carried out to female Latt (Wistar) of 8-20-week ** in the evening three days before injection, and abdominal administration of the hCG (Homo sapiens placenta nature Gonatrophin) was carried out in the evening the two days after. Following this, it put one male Latt (Wistar) of 8-20-week ** at a time into the gage, and mating was started. The vaginal

plug was inspected during the morning of the next day of mating, and female Latt who has checked the vaginal plug was moved to the Whitten culture medium which isolated and carried out HIARURONITAZE addition of the uterine tube after slaughter by cervical-vertebra dislocation. The egg was made to discharge from an uterine tube, and the fertilized egg was separated and washed under the stereoscopic microscope. 5-30 fertilized eggs were moved to the inverted microscope with differential interference equipment (NOMARU skiing equipment) at the culture medium drop on a hole vacancy slide glass using the system which combined the manipulator, and the microinjection of the DNA solution of 2pL(s) containing the DNA fragment prepared by the above of about 2,000 copies per fertilized egg was carried out to male pronucleus. The egg which DNA impregnation ended was cultivated until it transplanted to the oviduct. Transplantation parts differed in the developmental stage of a germ, the germ of 1 - 2 cell term was transplanted into the oviduct, and the germ of a 8 cell term - blastocyst term was transplanted into the uterus.

[0078]

In advance of embryo transfer actuation, activation of the corpus luteum of a recipient scalpel was performed and induction of the pseudopregnancy was carried out. That is, infertile copulation was carried out to the male which carried out vasoligature of Metz of a proestrus. The delivery scheduled day of a transplantation germ made the 1st day the day which the vaginal plug of a recipient scalpel attached, and calculated it as the 20th day. When transplanting into an oviduct, the germ of 1 cell term and 2 cell terms was transplanted under the stereoscopic microscope in the oviduct of the recipient rat of a pseudopregnancy day eye under the Nembutal anesthesia. About ten germs per single-sided oviduct were transplanted. When transplanting in a uterus, the germ which made it generate from a morula term by explantation at a blastocyst term was transplanted in the uterus of the recipient rat which carried out induction of the pseudopregnancy under the Nembutal anesthesia. The pseudopregnancy age in day of a recipient rat was calculated more youthfully [for one day] than the age in day of a germ. the case where judged the number of fetuses from appearance and the number of fetuses is expected to be five or more animals -- a natural birth -- the cesarean section was performed when four or less animals became. Born Latt separated from parents between 3-4 weeks of after the birth, and divided and bred the sex.

[0079]

Selection of a [example 3] transgenic rat

A part of tail was cut after four week ** of after the birth, and genomic DNA was extracted using the kit (Qiagen tissue kit; Qiagen). This was made into mold and magnification by PCR of an introductory gene fragment was performed. In magnification, it is CMV-F1 primer (5 '-GTC GAC ATT GAT TAT TGA CTA G -3' / array number: 9). CMV-R1 primer (5 '-CCA TAA GGT CAT GTA CTG -3' / array number: 10), Beta-gl -3 primer () [5'-CTT CTG] GCG TGT GAC CGG CG-3' / array number : 11 and two to hM2 primer (5 '-ATC GAA TTC TGA GAT CAT AAT CCC TGT GGG ATG C -3' / array number: 12), And eight to hM1 primer () [5'-TTA] TTC AGT GGC AAA GTT TCT TGC CCT TGA-3' / array number : Three pairs of primers of 13 and a beta-globinR primer (5 '-TCG AGG GAT CTT CAT AAG AGA AGA G -3' / array number: 14) are used. The individual from which a magnification product is acquired by all PCR by three pairs of primers was sorted out. Obtained F0 generation was made to cross with a normal individual (Wistar), F1 generation was obtained, F1 comrades of a hetero were multiplied further and F2 was obtained.

[0080]
[Example 4] Homo sapiens Meg Singh gene expression analysis
Northern blot analysis of a Homo sapiens Meg Singh gene was performed as it was the following. By random DNA labeling, RI indicator of the Bgl II/BamH I fragment of pBsCAG-2 which contain a Homo sapiens Meg Singh gene as an insertion was carried out, and it was used as the probe. This fragment corresponds near [poly A signal] a vector. All RNA (10microg) extracted from Latt's kidney mesangial cell was separated by 1% agarose gel containing 2.2 M formaldehyde, and it imprinted to the nitrocellulose filter. The filter was made to hybridize in a high buri solution. After hybridization, the last stringency of SDS washed 0.1xSSC / 0.1% at 55 degrees C.
Latt who used for the experiment is a transgenic rat and normal Latt (non-transgenic rat) of a brood.
The obtained result was shown in drawing 2 - drawing 4 . As for the manifestation comparison according to organ, drawing 3 , and 4, drawing 2 shows the result of a manifestation comparison of the hetero in the kidney and liver, and a gay. In the transgenic rat, it has checked that the Homo sapiens Meg Singh gene which is a foreign gene was strongly discovered (drawing 2). Moreover, it could check that the amount of manifestations was increasing the gay individual compared with a hetero individual, and it became clear that a manifestation with the kidney is stronger than liver (drawing 3 and drawing 4).
[0081]
[Example 5] Western blot analysis
The HITOMEGUSHIN manifestation of a transgenic rat was checked by the Western blot analysis of the organization extract which used the rabbit anti-HITOMEGUSHIN peptide antibody.
The organization sample (10microg) was homogenized by 100micro (pH6.8) of 0.35 tris hydrochloric acids L containing 10% of SDS, 36% of glycerol, 5% of beta-mercaptoethanol, and 0.012% of bromphenol blue of M, and carried out centrifugal separation for 10 minutes by 15,000g. The rabbit anti-HITOMEGUSHIN peptide IgG (5microg/mL) was used as a primary antibody, the alkaline phosphatase joint goat anti-rabbit IgG (Cappel) was used as a second antibody, and Meg Singh protein was detected. As positive control, purification recombination Meg Singh who was discovered in the CHO cell was used. The anti-actin antibody (sigma) was used as a negative control.
The manifestation of the Meg Singh protein was comparatively high at the kidney, the heart, and the pancreas, and low in a brain and liver (drawing 5).
[0082]
Production of a [example 6] anti-MEGUSHIN antibody
The rabbit anti-HITOMEGUSHIN polyclonal antibody was produced by the conventional approach. The monoclonal antibody to Meg Singh for immunity organization dyeing was produced by the following approach. Immunity of recombination Meg Singh of the CHO cell origin was carried out to the Balb/c mouse. The cell fusion of the spleen cell of a mouse and SP2/0 cell (mouse myeloma cell stock) which carried out immunity was carried out, and the Meg Singh antibody was screened about the supernatant liquid of the obtained hybridoma using enzyme immunoassay (ELISA). That is, the coat of the polystyrene ELISA plate (Nalge Nunc International) of 96 wells was carried out by Meg Singh (100 ng/well), and it incubated at the room temperature under 1 hour and 25% of existence of block ace (Dainippon Pharmaceutical) content Dulbecco's PBS (D-PBS).

By 0.05% of Tween20 content D-PBS (D-PBST), after washing a well, 100micro of supernatant liquid L of each hybridoma was added to each well. The well was added by D-PBST, the peroxidase-labeling goat anti-mouse IgG antibody (Chemicon) was added at the room temperature after washing for 2 hours, and it incubated at the room temperature for 2 hours. It incubated to the Mckvaine buffer (pH5.0) after adding to a well, and it was made to color the solution containing o-phenylenediamine (Nacalai Tesque) of 0.006% of H2O2 (Wako Pure Chem industry) and 0.4 mg/mL. The absorbance in 492nm was measured using the microplate reader (V-max:Molecular Device). About the hybridoma which was a positivity in ELISA, cloning was carried out with the extra dilution method.

[0083]

Immunity organization dyeing to the kidney tissue using a [example 7] MEGUSHIN peptide antibody (immuno HISUTOKE Myst)

MEGUSHIN peptide -2 antibody was prepared by the well-known approach (Inagi, R.et al.Biochem.Biophys.Res.Commun., 286, 1098-106, 2001).

Kidney tissue was extracted from Meg Singh TRANS GENIC Latt. Kidney tissue did embedding using freezing organization embedding agents (OCT compound) according to the conventional method. The 4-micrometer frozen section was produced using the FURIO stat from this freezing embedding organization. This frozen section was mounted on the slide which carried out the coat by 3-aminopropyl triethoxysilane (product made from a sigma) (4% paraformaldehyde immobilization, 15 minutes).

The frozen section was washed by PBS containing 0.5% of Tween20, and one evening incubated with anti-MEGUSHIN peptide -2 antibody after blocking and within the 4-degree C humidification chamber with 4% of skim milk. The organization intercept was washed and it incubated at the room temperature for 2 hours using the peroxidase-labeling goat anti-rabbit IgG antibody (product made from DAKO) diluted to 1:100. The 3 and 3'-diaminobenzidine solution containing 0.003% of hydrogen peroxide solution was used for detection of a peroxidase. The nucleus was dyed by the hematoxylin. A hematoxylin / eosine dyeing was carried out by the well-known approach.

The microphotography (NIKON ECLIPSE E400) which carried out tissue immunity dyeing to Meg Singh TRANS GENIC Latt's kidney tissue is shown in drawing 6 - drawing 8 . The kidney of a transgenic rat reacted with the HITOMEGUSHIN antibody in all fields, and the PAS positivity matter especially accepted in a part of glomerulus epithelial cell and renal tubule epithelial cell (distal tubule) presented the Tsuguaki positive stain so that clearly from drawing.

[0084]

Measurement of the inside of a [example 8] blood serum, and urine medium maturing chemistry data

Meg Singh TRANS GENIC Latt of this invention presents completely different phenotype from the Meg Singh TRANS GENIC mouse (patent reference 3 reference) already known. That was checked experimentally. The following items were measured about wild type Latt, Meg Singh TRANS GENIC Latt (gay), and the Meg Singh TRANS GENIC mouse.

Blood serum: Total protein (TP:Biuret law), total cholesterol (Tcho: enzymatic process), an urea nitrogen (BUN: urease UV method), a creatinine (Cr: alkali picric acid method), Meg Singh (the ELISA method).

Urine (24 hour urine collection): Total protein (TP: the pyrogallol red method), an urea

nitrogen (BUN: urease UV method), a creatinine (Cr: alkali picric acid method), sodium (Na: electrode method), Meg Singh (the ELISA method).

[0085]

In addition, measurement of Meg Singh in a blood serum was performed as follows. Heparin blood collecting was performed from Meg Singh TRANS GENIC Latt, and the after [centrifugal] blood serum was separated. the rabbit polyclonal anti-MEGUSHIN antibody (IgG fraction) which is an antibody for solid phase -- 2microg/ml -- diluting -- each well of the plate for 96 hole ELISA (F96 MAXSOPRP NUNC-IMMUNOPLATE: NUNC) -- every [100microL/well] -- it added and overnight neglect was carried out at 4 degrees C. Washing buffer solution: It washed by the Tween20 content PBS (-) and (Tween-PBS) 0.05% (w/v), and the block ace (Dainippon Pharmaceutical) was added 350microl/well, and was blocked for room temperature 1 hour. 100microL/well distributive pouring was carried out and Homo sapiens recombinant purification Meg Singh or the blood serum specimen which carried out phase dilution was made to react after washing with the washing buffer solution for room temperature 2 hours. the mouse monoclonal anti-MEGUSHIN antibody (IgG fraction) which are after washing, next an antibody for detection in the washing buffer solution -- 1microg/ml -- diluting -- every [100microL/well] -- it was made to react after addition for room temperature 2 hours The ALP indicator anti-mouse IgG antibody (chemicon) was added 100microL/well after washing, and it was left at the room temperature for 2 hours. after washing and a p-Nitrophenyl phosphate chromophoric substrate solution (SIGMA) -- every [100microL/well] -- 3 Ns [after making it react for 30 minutes at a room temperature in addition] NaOH -- every [100microL/well] -- in addition, the reaction was stopped, the absorbance (405nm) was measured with the microplate reader (the product made from a Japanese molecular device, SPECTRAmax250), and it asked for the Meg Singh concentration in a blood serum from the calibration curve of the standard solution.

A measurement result is shown in Table 1 and 2.

[0086]

[Table 1]

血清生化学データ （平均±標準偏差）

	週齢 (週)	体重 (g)	TP (g/dL)	BUN (mg/dL)	Cr (mg/dL)	メグシン (μg/mL)
野生型(n=14)	7.4±1.2	205±47	6.06±0.68	15.6±1.8	0.21±0.10	ND
ホモ (n=12)	6.9±0.8	43±7	4.61±0.74	54.0±23.5	0.32±0.34	157±32

[0087]

[Table 2]

尿中生化学データ （平均±標準偏差）

	週齢 (週)	TP (g/dL)	Cr (mg/dL)	TP/Cr (g/mL)
野生型(n=14)	7.4±1.2	37±38	42.5±16.5	0.87
ホモ (n=12)	6.9±0.8	396±564	13.6±9.6	29.1

[0088]
Carrying out the natural onset of the mesangial proliferative glomerulonephritis (kidney function normal) is known for the Meg Singh TRANS GENIC mouse (WO 01/24628). On the other hand, in Meg Singh TRANS GENIC Latt, it became clear to present a renal failure Mr. lesion (albuminuria) in youth from the inside of a blood serum and urine medium maturing chemistry data. Especially, as compared with Latt of a wild type, by the gay, serum creatinine/weight was rising 7.3 times, and presented the renal failure condition. Moreover, by Latt (especially gay), hypoplasia was accepted to that which carries out normal growth of the mouse (WO 01/24628).
[0089]
PAS stain of the [example 9] Meg Singh TRANS GENIC Latt kidney: Drawing 9 - drawing 11
The kidney was extracted from Latt and the PAS stain (periodic acid-Schiff stain) of mesangium was performed after cull NOR immobilization. Abnormalities were not accepted in the individual of a wild type. In the individual of a hetero, it applies to a glomerulus epithelial cell, a proximal tubule a distal tubule - a manifold, and is PAS. The deposition of the electropositive matter was accepted. The PAS same in a gay's individual on the other hand as a hetero The deposition of the electropositive matter was seen more seriously than a hetero and broadly. However, the view which shows glomerulus failures, such as glomerulosclerosis and fibrosis of stromata, was not accepted.
[0090]
Measurement of the inside of [example 10] plasma, and the glucose in urine
About the 9-weeks old Latt plasma and urine, it measured using glucose CII and Test Wako (Wako Pure Chem Industries). In 20micro of samples L which diluted Latt's plasma or urine 4 times with water, added 150micro of color reagents L, it was made to react for 15 minutes at a room temperature, the absorbance in 505nm was measured with the plate reader, and glucose concentration was computed from the standard curve.
A result is shown in Table 3. Meg Singh TRANS GENIC Latt presented hyperglycemia, and it was checked that it is useful as a diabetes-mellitus model animal.
[0091]
[Table 3]
Inside of plasma, and glucose concentration in urine (average ** standard deviation)
Week-old Glucose in plasma Glucose in urine
(Week) (mg/dL) (ng/mL)
Wild type (n= 14) 7.4**1.2 139**31 11**4
Gay (n= 12) 6.9**0.8 676**175 566**110
[0092]
Hematoxylin eosin staining of the [example 11] Meg Singh TRANS GENIC Latt pancreas
Hematoxylin eosin staining of the Latt pancreas was performed with the conventional method. consequently -- Meg Singh TRANS GENIC Latt (gay) -- many Langerhans' islets -- a blank cartridge -- although denaturation was caused, most infiltration of an inflammatory cell was not accepted. By some parts, it leaves pancreas parenchyma, withering of stromata is conspicuous, the RA Mr. islet cell decreased, and infiltration of neovascularity or fibrocyte was seen. When it furthermore went on, it was occupied by fibrous connective tissue or the glass Mr. object, and the RA Mr. islet cell disappeared (

drawing 12).

[0093]

Measurement of the insulin in [example 12] plasma

5micro of 9-weeks old Latt plasma L was measured using the MORINAGA super-high sensitivity rat insulin measurement kit (Morinaga student science laboratory) of the ELISA method. Make 5micro of Latt plasma L, and 95micro of specimen diluents L react to the solid phase-ized anti-rat insulin monoclonal antibody on a plate, next made 100micro [of enzyme-labeling guinea pig anti-rat insulin antibodies] I react, it was made to color by 100micro of enzyme substrate solutions L, 100micro of stop solutions L was added, and the rat insulin concentration to the absorbance obtained from a plate reader's wavelength of 450nm was computed from the standard curve.

A result is shown in Table 4. It was checked that Meg Singh TRANS GENIC Latt's insulin concentration in plasma is intentionally low compared with a wild type.

[0094]

[Table 4]

Insulin concentration in plasma (ng/mL, average ** standard deviation)

Week-old Insulin concentration

(Week) (ng/mL)

Wild type (n= 14) 7.4**1.2 3.6**2.8

Gay (n= 12) 6.9**0.8 < 0.1

[0095]

The comparison of the model rat of this invention and the Meg Singh TRANS GENIC mouse (patent reference 2 and 3 and nonpatent literature 26 reference) in each measured value is shown below.

[0096]

[Table 5]

	本発明のモデルラット	メグシントランスジェニックマウス
特　徴	腎不全（腎機能低下、蛋白尿）、糖尿病を自然発症	メサンギウム増殖性糸球体腎炎を自然発症
腎機能	低下（蛋白尿、糸球体障害なし）	正常（糸球体障害あり）
発症齢	６週齢（腎不全） ９週齢（糖尿病）	３５～４０週齢
発症率	１００％	４０～６０％
性　差	な　し	な　し
発　育	発育障害 雌雄とも同じ体重推移（正常個体は雌雄差あり）、体長短い・痩せ	正常発育
病理像	糸球体上皮、遠位尿細管上皮～集合管、膵臓にPAS陽性沈着物（メサンギウム領域には認めない）、ランゲルハンス島全体、膵臓外分泌部の一部の腺房に変性	メサンギウム基質領域の増生、免疫複合体の沈着およびメサンギウム細細胞の増加を伴う典型的なメサンギウム細胞増殖性腎炎症状。
メグシンタンパク	腎臓に多い	腎臓に多い
生化学データ	血清 TP 低（正常の 75%：7 週齢） 血清 Tcho 高 （正常の 2.2 倍：7 週齢） BUN 高（正常の 3.5 倍：7 週齢） 血清クレアチニン/体重高 （正常の 3.4 倍：7 週齢） 尿 TP 高（正常の 11 倍：7 週齢） Ccr 低（正常の 1/4：7 週齢） 血糖値高（正常の 5 倍：7 週齢）	40 週齢で全て正常範囲内

[0097]
[Example 13] action trial
9-weeks old Meg Singh TRANS GENIC Latt showed a remarkable non-recognizing sexual behavior change. In the half-enclosure ramp trial, the clear ataxic walk was observed as compared with the un-transgenic litter.
[0098]
MAP2 and Caspase3 immunity organization dyeing of a [example 14] hippocampus field The organization of a hippocampus field was extracted from Meg Singh TRANS GENIC Latt. The organization did embedding using freezing organization embedding agents (OCT compound) according to the conventional method. The 4-micrometer frozen section was produced using the FURIO stat from this freezing embedding organization. This frozen section was mounted on the slide which carried out the coat by 3-aminopropyl

triethoxysilane (product made from a sigma) (4% paraformaldehyde immobilization, 15 minutes).
It washed by PBS containing 0.5% of Tween20, one evening incubated with anti-MAP2 antibody or anti-Caspase3 antibody after blocking and within the 4-degree C humidification chamber with 4% of skim milk, and the frozen section was dyed with the conventional method.
The microphotography (NIKON ECLIPSE E400) which carried out immunity organization dyeing to the organization of Meg Singh TRANS GENIC Latt's hippocampus field is shown in drawing 13 . MAP2 which is one of the microtubule related protein in all the fields of Meg Singh TRANS GENIC Latt's hippocampus had almost disappeared, and the afunction of the nerve cell of a hippocampus field was suggested so that clearly from drawing. Moreover, dyeing of Caspase3 was accelerating remarkably. By Meg Singh TRANS GENIC Latt, it became clear from these things that the nerve cell death by apotosis is accelerating in a hippocampus field.
[0099]
[Example 15] study trial
(1) Use animal
8-weeks old Meg Singh TRANS GENIC Latt (gay type) (two males, four females) and wild type Latt (two males, four females) were used. Each rat was bred at the breeding room set as the room temperature of 20-26 degrees C, 35 - 75% of humidity, and 12 hours (7:00- 19:00)/day of lighting time amount, and carried out free intake of a cubed diet F-2 (Funabashi farm) and the tap water.
[0100]
(2) Passive evasion trial (light and darkness Box)
The step-through mold passive avoidance reaction experimental device (light and darkness Box and a timer: a lab support, shock generator:medical treatment agent) divided into **** and a dark room was used. When an animal was put into a ** room as acquisition trial and it shifted to a dark room, electrical stimulation (100V, 6mA, and 60Hz (set point)) was given. Acquisition trial considered as one day, and it was performed until latent time reached at 300 seconds (0 day). On the other hand, treatment same [without giving electrical stimulation as maintenance trial at the next day (1 day)] was performed, and time amount until it shifts to a dark room was measured as reaction latency (second). Reaction latency could be a maximum of 300 seconds. The reaction latency of maintenance trial and the count of trial in acquisition trial (count of [stimulated]) were measured with the acquisition trial list.
[0101]
(3) Statistical art
The test result was displayed by the average ** standard error (Mean**S.E.). Significant difference assay between groups performed chi2 assay in weight and the acquisition trial list in a passive evasion trial about the count [in / reaction latency / of maintenance trial / for the t test of Student / acquisition trial] of trial (count of [stimulated]). The SAS System Release 8.2(TS2MO) for Windows (TM) (SAS Institute) and its interlocking system (EXSAS, Ver.6.10, arm) were used for assay.
[0102]
(4) Passive evasion test result (light and darkness Box)
A test result is shown in Table 6. In acquisition trial, the count of trial to acquisition (count of [stimulated]) increased the transgenic rat group by intentionally for latent time 300 seconds compared with the wildness group. Moreover, significant extension

was accepted also in the reaction latency of the 2nd acquisition trial. In playback trial, significant compaction was accepted in the reaction latency on the 1st. From the above result, Meg Singh TRANS GENIC Latt (gay type) is inferior to the acquisition list in study in the maintenance ability compared with the wild type, and is judged to have a study memory disorder.

[0103]

[Table 6]

	動物No.		体重(g)	獲得試行 潜時(秒) 1回	2回	3回	4回	5回	6回	試行回数 (被刺激回数)	保持試行 潜時(秒) 1day
野生型	101	雄	249.2	103.3	300	300	300	300	300	1	300
	102	雄	238	64.1	300	300	300	300	300	1	300
	103	雌	177.4	31.5	300	300	300	300	300	1	110.2
	104	雌	170.8	40.6	300	300	300	300	300	1	300
	105	雌	192.8	12.7	300	300	300	300	300	1	300
	106	雌	192.7	19.6	300	300	300	300	300	1	300
	平均		203.5	45.3	300	300	300	300	300	1	268.4
	S.E.		13.2	13.7	0	0	0	0	0	0	31.6
ホモ型	201	雄	62.8	28.3	6.3	29.7	105.7	105.4	300	5	87.3
	202	雄	130.5	95.1	119.7	300	300	300	300	2	42.3
	203	雌	70.3	13.8	60.5	300	300	300	300	2	死亡
	204	雌	70.2	30.1	300	300	300	300	300	1	46.3
	205	雌	65.6	45.6	55.6	300	300	300	300	2	死亡
	206	雌	89.3	試験せず	試験せず	試験せず	試験せず	試験せず	試験せず	試験せず	死亡
	平均		81.5	42.6	108.4	205	217.6	217.6	300	2.4	58.6
	S.E.		10.5	14.1	51.2	60.2	53.9	53.9	0	0.7	14.4
			＊＊		＊＊					＊	＊＊

＊:P<0.05
＊＊:P<0.01

[Availability on industry]
[0104]
By this invention, the animal used in disease modeling by installation of the Meg Singh gene was offered. The animal of a certain kind which reinforced the manifestation of Meg Singh in the kidney presents a rise and albuminuria of the creatinine value in blood. Furthermore, this animal shows the failure applied to a glomerulus epithelial cell, a proximal tubule a distal tubule - a manifold as a characteristic pathology image. Such a view has proved that the animal used in disease modeling of this invention is presenting the symptoms more near human renal failure. Therefore, it is considered utility by the cause elucidation of renal failure.
The analysis of the onset mechanism of renal failure or symptoms is attained using the model animal of this invention. Moreover, the model animal of this invention is useful to development of the remedy of renal failure, screening, and a pan because of assay of drugs etc.
[0105]
Furthermore, this invention realized offer of the animal used in disease modeling accompanied by a hyperglycemia symptom. Although the model animal accompanied by hyperglycemia is known, the animal which can bring about a hyperglycemia condition certainly for a short period of time is not known like the animal used in disease modeling of this invention. The animal used in disease modeling of this invention is useful to the elucidation of the various analyses of symptoms and the retrieval of the therapy approach resulting from hyperglycemia, and the therapy approach of hyperglycemia. It is shown clearly that hyperglycemia is the cause which causes various failures. Therefore, the model animal which can observe various morbid change brought about with hyperglycemia is useful in the symptoms analysis.
The symptoms which the animal used in disease modeling of this invention presents are in agreement with the symptom looked at by type 1 diabetes. Therefore, the animal used in disease modeling of this invention is useful as a model animal of insulin dependent diabetes mellitus.
Moreover, this invention offered the model animal of a neurodegenerative disease. Offer of the neurodegenerative animal used in disease modeling which presents neurodegenerative [which was stabilized with high repeatability] by this invention was attained. The neurodegenerative animal used in disease modeling offered by this invention is very important when you understand the pathophysiology of the disease of a nervous system.
[Brief Description of the Drawings]
[0106]
[Drawing 1] It is drawing showing the construction Fig. of a recombination gene construct. A shows the structure of pBsCAG-2. B shows the structure of the DNA fragment used for pBsCAG2/Megsin and the microinjection to an egg.
[Drawing 2] The photograph in which the detection of Meg Singh mRNA in the various organs by the Northern blot of wild type Latt and the model rat (hetero) of this invention is shown.
[Drawing 3] The photograph in which the result of the Northern blot which analyzed the Homo sapiens Meg Singh gene expression in the Latt kidney is shown. A wild type and lanes 3 and 4 belong [a hetero and the lanes 5 and 6 of lanes 1 and 2] to a gay.
[Drawing 4] The photograph in which the result of the Northern blot which analyzed the

Homo sapiens Meg Singh gene expression in the Latt liver is shown. A wild type and lanes 3 and 4 belong [a hetero and the lane 5 of lanes 1 and 2] to a gay.
[Drawing 5] The photograph in which the result of Western blot which analyzed Latt's (a wild type, gay) HITOMEGUSHIN manifestation is shown.
[Drawing 6] The photograph in which immunity organization dyeing to Meg Singh in the Latt (wild type) kidney organization is shown (100 times).
[Drawing 7] The photograph in which immunity organization dyeing to Meg Singh in the Latt (hetero) kidney organization is shown (25 times).
[Drawing 8] The photograph in which immunity organization dyeing to Meg Singh in the Latt (hetero) kidney organization is shown (50 times).
[Drawing 9] The photograph in which the PAS stain of the Latt (wild type, 9 weeks old) kidney organization is shown (400 times).
[Drawing 10] The photograph in which the PAS stain of the Latt (hetero, 9 weeks old) kidney organization is shown (400 times).
[Drawing 11] The photograph in which the PAS stain of the Latt (gay, 9 weeks old) kidney organization is shown (400 times).
[Drawing 12] The photograph in which hematoxylin eosin staining of the Latt (wild type, gay, 9 weeks old) kidney organization is shown (400 times).
[Drawing 13] The microphotography in which the result which carried out immunity organization dyeing to the organization of Meg Singh TRANS GENIC Latt's hippocampus field is shown (400 times).

[Translation done.]

DESCRIPTION OF DRAWINGS

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